

Some Effects of Nitrogen-fixing Bacteria on the Growth of Non-Leguminous Plants.

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During the course of an investigation on "The Cross-inoculation of the nodule-forming bacteria from Leguminous and non-Leguminous plants,"* it was noticed that in all the bacterial cultures prepared from the algal zone of the root-tubercles of cycads taken from below the surface of the soil, *Pseudomonas radiculicola* was associated with a species of *Azotobacter*.

In order to determine to what extent, if any, this association gave an increased power of assimilating free nitrogen, the two forms were obtained as pure cultures by successive platings on a medium composed of maltose 20 grammes, monobasic potassium phosphate 0·5 gramme, sodium chloride 0·5 gramme, calcium carbonate 0·5 gramme, ferrous sulphate 0·1 gramme, agar 15 grammes, and distilled water 1000 c.c. Separate cultures of each and a mixed culture were then grown in fluid media in duplicate 500 c.c. Erlenmeyer flasks containing 250 c.c. of the above medium, omitting the agar but adding 10 grammes of mannite. Control flasks were kept side by side with the inoculated flasks.

All the flasks were incubated at 24° C. for 15 days, care being taken to renew the air in the flasks at intervals, then a nitrogen determination was made of the contents of each flask. The results of these analyses gave the following averages:—

Control	0·48	milligramme	N per 100 c.c.
<i>Pseudomonas</i> alone.....	0·91	„	„
<i>Pseudomonas</i> + <i>Azotobacter</i> ...	1·24	„	„

Hence *Pseudomonas* and *Azotobacter* together make a powerful combination for the fixation of free nitrogen.

The bacteria in the root-tubercles of cycads appear to live imbedded in the slime they produce *outside* the cortical cells in the open spaces of the so-called algal zone. The cortical cells which project into this zone presumably absorb the nitrogenous products of the bacterial activity, and thus the cycad is benefited. If, therefore, this combination of bacteria *outside* the cortical cells is a direct benefit to the cycas plant, the possibility presented itself that

* Report British Association, 1907.

a mixed culture of *Pseudomonas* and *Azotobacter* applied directly to the roots of other non-leguminous plants might benefit their growth also.

The first experiments were made on oats (*Avena sativa*). Four 5-inch pots were filled with sand freed as far as possible from organic matter and nitrates, and given a sufficient dressing of phosphates, potash, and lime. Twenty oat seeds were planted in each pot, and as soon as the young plants were about 1 inch high two of the pots were watered with a mixed culture of *Pseudomonas* and *Azotobacter* grown in the previously mentioned medium. The plants were watered regularly with distilled water and allowed to grow until the untreated plants exhibited signs of drooping. Each plant was then carefully air dried and weighed, with the following result:—

Untreated, average weight per plant	0.42 gramme
Treated	0.74 „

an increase of 0.32 gramme, or 76 per cent.

In 1908, field experiments were made on barley. Two plots, each having an area of 484 square yards, were planted, one with seed moistened with the mixed culture, the other with seed untreated. The land was very poor and low in organic nitrogen, having carried oats in 1906 and barley in 1907. The yield of grain per plot at harvest was:—

Untreated.....	608 lb.
Treated.....	691 „

an increase of 83 lb., or 13.6 per cent.

On the same farm a strip of land was sown with treated seed through the centre of a 34-acre barley field. It was found impossible at harvest to keep the yield of treated seed separate from the rest, but samples of grain from the treated and untreated parts were taken and analysed, and it was found that the barley from the treated seed had the higher nitrogen-content.

	Milligrammes of N per cent.	Weight of 1000 corns.	Milligramme N per corn.
Untreated.....	1.55	48.5	0.75
Treated.....	1.76	49.5	0.87

Experiments on hyacinths (*Galtonia candicans*) grown in sandy soil. The soil was dressed with lime in August, 1907, and remained fallow until April, 1908, when it was manured with cow manure—10 tons to the acre. Bulbs of equal size were then planted, 250 in each bed, a path of 14 inches dividing the two beds. The treated bulbs were twice watered with mixed culture solution, once in May and once in June, the control bed being watered with pure water at the same time. The difference between the treated and

untreated bulbs was very noticeable during growth, the treated being more vigorous. The treated bulbs, when lifted, were noticeably larger than the untreated. After the bulbs were dry they were carefully weighed, and yielded

Untreated.....	69 lb. 3 oz.
Treated	82 „ 1½ „

an increase of 12 lb. 14½ oz., or 18·6 per cent.

Experiments on parsnips grown in ordinary garden soil. In January, 1908, the ground was deeply dug and given a medium dressing of London dung followed by a dressing of powdered chalk. The seeds were sown early in February in rows running north and south. A fortnight after the north half of each row was watered with a mixed culture of *Pseudomonas* and *Azotobacter*. In January, 1909, the roots were harvested, *every* root being weighed, with the following results:—

Untreated	68 roots	22 lb. 14 oz.	5·38 oz., average per root
Treated	65 „	26 „ 10 „	6·55 „ „

an increase of 21·7 per cent.

For the fixation of free nitrogen in laboratory cultures of *Pseudomonas* and *Azotobacter* the presence of carbonate of lime in the medium is necessary. In all the above experiments care was taken that a sufficiency of carbonate of lime was present in the soil to enable the bacteria to do their work effectively.
